Effects of combination treatment with cabozantinib and bortezomib in the 5TGM1 murine multiple myeloma model

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Introduction

Cabozantinib (cabo) is an inhibitor of tyrosine kinases including MET, VEGFR2, RET, and the TAM family kinases TYRO3, AXL, and MER, and has shown clinical activity in patients with castration-resistant prostate cancer and other solid tumors with bone metastases (1-3). Multiple myeloma (MM) is a monoclonal B-cell (plasma cell) neoplasia representing ~2% of all cancer deaths. The clinical hallmark is presence of multiple osteolytic lesions causing bone pain, pathologic fractures, and hypercalcemia. Circulating levels of HGF and VEGF are upregulated in MM patients (4), and regulation of plasma cell-osteoblast communication by the HGF-MET signaling pathway has been implicated in the development of lytic bone disease in these patients (5).

Aim of the Study

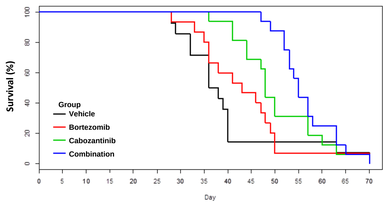
We have previously shown that cabo is active in the syngeneic 5TGM1 mouse MM model (6). This study aimed to determine whether combination with bortezomib would yield additional benefit.

Materials and Methods

Four experimental groups were included: control group receiving vehicle, bortezomib group (bz, 0.5 mg/kg ip twice a week), cabo group (10 mg/kg, PO QD) and combination group (cabo 10 mg/kg, PO QD + bz, 0.5 mg/kg ip twice a week). Female C57BL/KaLwRij mice, 6-7 weeks old, were allocated to treatment groups (n=15-16 per group) with equivalent average body weights. At day 0, animals were inoculated with 5TGM1 mouse myeloma cells by IV administration. Dosing began at day 1 and continued daily until euthanasia. Body weights were determined twice a week and blood samples were collected at days -1, 16, 23, 35 and at sacrifice for analysis of paraprotein IgG2b (ELISA kit, Bethyl Laboratories Inc, Montgomery, TX, USA) and tartrate-resistant acid phosphatase 5b (TRACP 5b; MouseTRAP kit, IDS, Boldon, UK). The development of osteolytic lesions was detected by radiography at day 35 and at sacrifice. Analgesia (buprenorphine 0.02 mg/ml in the drinking water) was used when signs of pain were observed. The mice were sacrificed when weight loss over 20%, paraplegia or breathing problems were observed, or at study day 70. Statistical analysis: biochemical markers up to day 35 was analyzed with LME model with day -1 values as baseline; osteolytic area with ANOVA followed by Tukey's HSD test; paraplegia resistance with Kruskal-Wallis followed by Mann-Whitney U-test; survival and time to first signs of paraplegia with log-rank test of Kaplan-Meier estimates.

Survival analysis

A



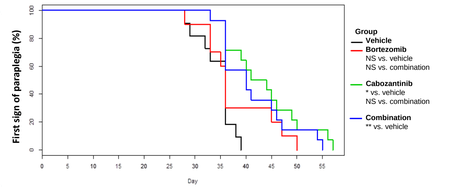
B

	Vehicle	Bortezomib	Cabozantinib	Combination
Median survival (days)	37	43	48	55
adj. p-value vs vehicle	BL	0.340	0.090	0.049
adj. p-value vs combination		0.005	0.280	BL
Hazard ratio vs vehicle	BL	0.70	0.47	0.42
95% CI vs vehicle	BL	0.331-1.475	0.221-0.997	0.197-0.882
Hazard ratio vs combination		3.16	1.64	BL
95% CI vs combination		1.477-6.762	0.815-3.303	BL

FIGURE 1 A) Kaplan-Meier curve of survival. **B)** Median survival and hazard ratios compared to vehicle and combination therapy. Cabo + bz combination therapy significantly increased overall survival. BL= baseline (comparator group).

Paraplegia

A



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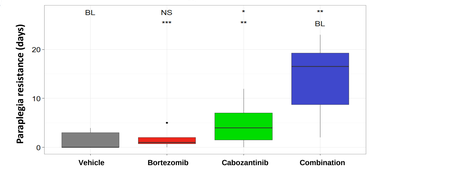

FIGURE 3. A) Kaplan-Meier curve of time to first signs of paraplegia. Cabo mono- and combination therapies delayed the occurrence of symptoms. Animals without paraplegia are excluded. **B)** Paraplegia resistance: time from first signs to full paraplegia. Cabo monotherapy increased the time to full paraplegia and the effect was stronger with combination therapy. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, NS = non-significant, BL= baseline (comparator group).

Biochemical markers

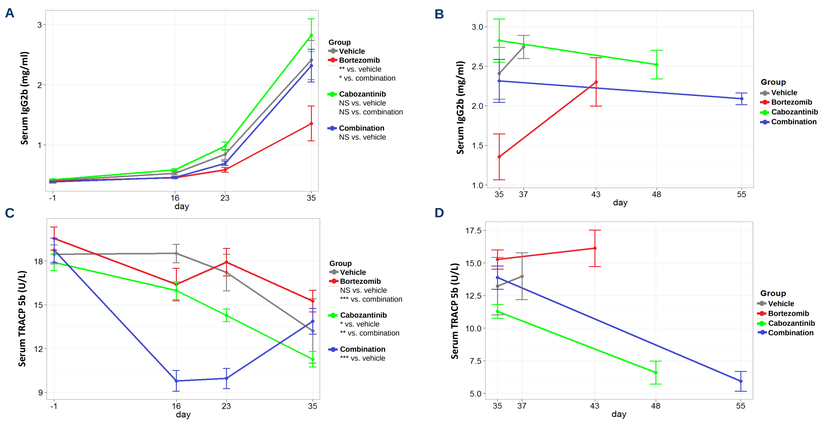

FIGURE 2. A) Secreted IgG2b in serum was measured as a tumor marker (mg/ml, mean±SEM). Bz decreased serum IgG2b levels compared to vehicle group. **B)** Change of serum IgG2b from day 35 to sacrifice. Median survival of the group is used as the sacrifice timepoint. IgG2b increased in vehicle and bz groups, but decreased in cabo and combination groups. **C)** Serum TRACP 5b was measured as marker of osteoclast number (U/L, mean±SEM). TRACP 5b levels decreased in cabo and combination groups compared to vehicle and in combination group compared to both monotherapies. **D)** Change of serum TRACP 5b from day 35 to sacrifice. Median survival of the group is used as the sacrifice timepoint. TRACP 5b increased slightly in vehicle and bz groups, but decreased substantially in cabo and combination groups. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, NS = non-significant, BL= baseline (comparator group).

Radiographic analysis

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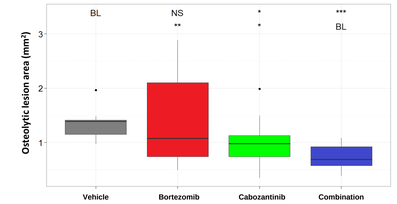

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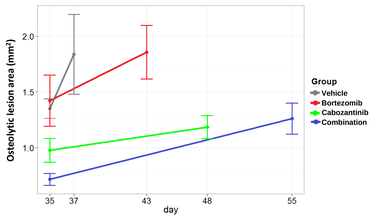

FIGURE 4. A) Total osteolytic area at day 35 was determined from X-ray images (mm^2, median±IQR25%±min/max). Outliers are marked as floating points in the figure but they were not removed in the statistical analysis. Cabo monotherapy decreased total osteolytic area, and the effect was more pronounced with combination therapy. **B)** Development of total osteolytic area from day 35 to sacrifice (mean±SEM). Median survival of the group is used as the sacrifice timepoint. Statistical analysis was not performed, but osteolytic lesion area appears to stay lower in both cabo treated groups also at sacrifice. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, NS = non-significant, BL= baseline (comparator group).

Summary

- The combination treatment of cabo + bz resulted in significantly improved overall survival, whereas both monotherapies resulted in non-significant trends for improved survival.
- Osteolytic lesions were reduced by cabo alone and further reduced by the combination, but were not affected by bz alone.
- By day 35, only bz monotherapy had inhibited the rise in serum IgG2b levels, whereas no change was observed in mice treated with the combination.
- An early rise in IgG2b followed by a reduction later in treatment was observed with cabo monotherapy. This pattern may be due to lysis of plasma cells, and is consistent with our previous observations that cabo dose-dependently increased the necrotic tumor area in bone.
- Cabo mono- and combination therapies delayed both the first symptoms of paraplegia and progression to full paraplegia.

Conclusions

The combination of cabozantinib + bortezomib significantly improved overall survival and exhibited bone-protective and anti-tumor effects in this murine model of MM.

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